Adiponectin expression in patients with inflammatory cardiomyopathy indicates favourable outcome and inflammation control

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Abstract: Aims Circulating adiponectin (APN) is an immunomodulatory, pro-angiogenic, and anti-apoptotic adipocytokine protecting against acute viral heart disease and preventing pathological remodelling after cardiac injury. The purpose of this study was to describe the regulation and effects of APN in patients with inflammatory cardiomyopathy (DCMi). Methods and results Adiponectin expression and outcome were assessed in 173 patients with DCMi, 30 patients with non-inflammatory DCM, and 30 controls. Mechanistic background of these findings was addressed in murine experimental autoimmune myocarditis (EAM), a model of human DCMi, and further elucidated in vitro. Adiponectin plasma concentrations were significantly higher in DCMi compared with DCM or controls, i.e. 6.8 ± 3.9 µg/mL vs. 5.4 ± 3.6 vs. 4.76 ± 2.5 µg/mL (P< 0.05, respectively) and correlated significantly with cardiac mononuclear infiltrates (CD3+: r2= 0.025, P= 0.038; CD45R0+: r2= 0.058, P= 0.018). At follow-up, DCMi patients with high APN levels showed significantly increased left ventricular ejection fraction improvement, decreased left ventricular end-diastolic diameter, and reduced cardiac inflammatory infiltrates compared with patients with low APN levels. A multivariate linear regression analysis implicated APN as an independent prognostic factor for inhibition of cardiac inflammation. In accordance with these findings in human DCMi, EAM mice exhibited elevated plasma APN. Adiponectin gene transfer led to significant downregulation of key inflammatory mediators promoting disease. Mechanistically, APN acted as a negative regulator of T cells by reducing antigen specific expansion (P< 0.01) and suppressed TNF -mediated NF B activation (P< 0.01) as well as release of reactive oxygen species in cardiomyocytes. Conclusion Our results implicate that APN acts as endogenously upregulated anti-inflammatory cytokine confining cardiac inflammation and progression in DCMi

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BASIC SCIENCE

Adiponectin expression in patients with inflammatory cardiomyopathy indicates favourable outcome and inflammation control

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Methods and results Adiponectin expression and outcome were assessed in 173 patients with DCMi, 30 patients with non-inflammatory DCM, and 30 controls. Mechanistic background of these findings was addressed in murine experimental autoimmune myocarditis (EAM), a model of human DCMi, and further elucidated *in vitro*. Adiponectin plasma concentrations were significantly higher in DCMi compared with DCM or controls, i.e. 6.8 $\pm$ 3.9 μg/mL vs. 5.4 $\pm$ 3.6 vs. 4.76 $\pm$ 2.5 μg/mL ($P < 0.05$, respectively) and correlated significantly with cardiac mononuclear infiltrates (CD3+: $r^2 = 0.025$, $P = 0.038$; CD45R0+: $r^2 = 0.058$, $P = 0.018$). At follow-up, DCMi patients with high APN levels showed significantly increased left ventricular ejection fraction improvement, decreased left ventricular end-diastolic diameter, and reduced cardiac inflammatory infiltrates compared with patients with low APN levels. A multivariate linear regression analysis implicated APN as an independent prognostic factor for inhibition of cardiac inflammation. In accordance with these findings in human DCMi, EAM mice exhibited elevated plasma APN. Adiponectin gene transfer led to significant downregulation of key inflammatory mediators promoting disease. Mechanistically, APN acted as a negative regulator of T cells by reducing antigen specific expansion ($P < 0.01$) and suppressed TNFα-mediated NFκB activation ($P < 0.01$) as well as release of reactive oxygen species in cardiomyocytes.

Conclusion Our results implicate that APN acts as endogenously upregulated anti-inflammatory cytokine confining cardiac inflammation and progression in DCMi.

Keywords Inflammatory cardiomyopathy • Adiponectin • Heart failure

Introduction

Adiponectin (APN), a 30 kDa adipocytokine, is constitutively present in high concentrations in human plasma as a low molecular weight (LMW), a middle molecular weight (MMW), and a high molecular weight (HMW) form. Adiponectin is mainly produced by adipose tissue but cardiomyocytes are also capable of synthesizing APN and express its receptors, APN receptor 1 (APN-R1) and APN receptor 2 (APN-R2).[1,2] Adiponectin exhibits immunomodulatory, anti-proliferative, anti-apoptotic, and pro-angiogenic effects and APN plasma concentrations are inversely correlated with classical cardiovascular risk factors such as body mass index (BMI),[3] hypertension, and diabetes. In animal

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Table 1 Baseline characteristics of patients

	Control, $n = 30$	DCM, $n = 30$	DCMi, $n = 173$
Sex (m/f)	17/13	27/3	139/34
Age (years)	44.03 ± 12.85	46.72 ± 13.48	51.13 ± 12.92
BMI (kg/m^2)	24.3 ± 4.36	26.96 ± 3.96	27.05 ± 4.91[a]
EF (%)	70.1 ± 8.07	38.57 ± 13.59	31.79 ± 13.55[a]
LVEDD (mm)	50.61 ± 6.27	59.64 ± 10.05	61.6 ± 10.02[a]
LVEDP (mmHg)	10.96 ± 3.9	14.8 ± 8.82	14.39 ± 8.78
PAP (mmHg)	12.96 ± 3.8	16.89 ± 8.12	22 ± 11.66[a]
PC (mmHg)	7.54 ± 3.56	9.58 ± 6.78	12.27 ± 9.03[a]
CI (L/min/m^2)	3.77 ± 0.84	3.39 ± 0.71	2.86 ± 0.7[a]
CD3+ (n/mm^2)	1.33 ± 1.14	1.5 ± 1.06	16.15 ± 9.14[a,b]
CD45RO (n/mm^2)	5.52 ± 2.7	4.42 ± 3.66	21.37 ± 31.22[a,b]
NYHA	II: 14; III: 16	II: 12; III: 18	II: 78; III: 95

Data are presents as mean ± SD.
[a]DCMi vs. control $P < 0.05$.
[b]DCMi vs. DCM $P < 0.05$.

studies, APN inhibited cardiac remodelling following aortic banding and myocardial infarction.[4,5]

Low concentrations of APN in humans have been associated with the increased risk of incident coronary heart disease in healthy participants.[6] High concentrations of APN have been associated with increased disease severity and adverse outcomes in patients with established heart failure, and possibly result from the cachexia observed in more advanced disease stages.[7–9] Although one cross-sectional study showed APN concentrations to be greater in patients with heart failure compared with controls, a prospective cohort study of elderly Swedish men and a recently published trial of the Framingham Offspring Study failed to detect an association between APN concentrations and heart failure incidence.[10] Therefore, the contradicting observations of APNs' cardiovascular effects clearly need further elucidation.

Inflammatory cardiomyopathy (DCMi) is frequently caused by viral infection that has been implicated as an important causal factor responsible for the progression to dilated cardiomyopathy. Viral infection triggers an inflammatory process, i.e. by expression of cytokines, chemokines, and abundant infiltration with T cells and macrophages that outlasts the initial replicative phase and may lead to postviral autoimmunity. Chronic inflammation leads to tissue injury, endothelial dysfunction, and cardiac remodelling resulting in deterioration of myocardial contractility and left ventricular ejection fraction (LVEF). Therefore, the immune response characterized by immunohistologically proven inflammatory infiltrates plays a crucial role for the outcome of DCMi.[11]

Adiponectin exerts profound immunomodulatory effects and modulates cytokine production and phagocytosis of macrophages[12] as well as cytotoxicity of NK cells.[13] Although initially described as anti-inflammatory cytokine, more recent studies also reported pro-inflammatory effects.[14,15] In this regard, APN was shown to induce NFκB *in vitro*, a master transcription factor for pro-inflammatory cytokines.[16] However, most animal studies provide evidence for an anti-inflammatory role. In a mouse model of encephalomyocarditis (EMC) virus-induced myocarditis, impaired expression of cardiac APN contributed to the progression of viral myocarditis.[17] In line with this finding, APN replacement therapy attenuated myocardial damage in leptin-deficient mice with acute viral myocarditis.[18] Furthermore, better survival of obese KKAy mice with viral myocarditis on a reduced energy diet correlated with higher cardiac APN expression.[19] Moreover, APN gene transfer ameliorated the degree of chronic inflammation by promoting the clearance of apoptotic bodies via calreticulin-dependent phagocytosis in lpr mice with lupus-like autoimmune disease.[20]

The effects of APN on inflammation in human DCMi have not been studied yet. Therefore, we investigated the systemic and cardiac APN expression of APN, its correlation with haemodynamic and inflammatory parameters in plasma and endomyocardial biopsy (EMB) specimens, and its effects on the outcome in well-characterized patients with DCMi. In order to extend our mechanistic insights and corroborate our findings, we performed additional *in vitro* and *vivo* experiments using a mouse model of experimental autoimmune myocarditis (EAM) that mimics certain aspects of human DCMi.

Methods

Patients

Two hundred thirty-three consecutive patients who were submitted with clinical symptoms and signs of heart failure (i.e., dyspnoea and chest pain, NYHA II-III) underwent right ventricular EMB for histological, immunohistological, and molecular virological analysis following angiographic and echocardiographic exclusion of coronary heart disease and other possible causes of cardiac dysfunction such as valvular or systemic diseases with cardiac involvement. Endomyocardial biopsies were assessed for inflammation and viral genomes using standardized protocols.[21] According to the protocol of the study, patients were divided into a control group, in which the diagnostic workup finally revealed that their complaints were non-cardiac in origin, a DCM and a DCMi group. At enrolment, the duration of observed symptoms was 34 ± 5.65 days. The following criteria defined control patients: LVEF >60%, left ventricular end-diastolic diameter (LVEDD) < 55 mm, CD3+ <7 per mm^2, ($n = 30$); patients with decreased LVEF <60%, increased LVEDD >55 mm, and a negative myocardial inflammation score (CD3+ <7 per mm^2) were included into a DCM group ($n = 30$). Inflammatory cardiomyopathy patients showed LVEF <60%, LVEDD >55 mm, and CD3 >7 per mm^2 ($n = 173$). Inflammatory cardiomyopathy was defined as a cardiomyopathy with decreased LVEF, increased LVEDD, and a positive myocardial inflammation score according to the Dallas criteria and criteria of the World Heart Federation (WHF). Demographic and clinical characteristics of patients are shown in *Table 1*. A follow-up examination of all patients in the DCMi group for determination of LVEF by an echocardiographic study was performed 6 months after inclusion into the study registry. All patients were treated according to the guidelines for medical treatment of heart failure (Supplementary material online, *Table S1*). Moreover, 63 patients with persistent symptoms of heart failure despite heart failure medication underwent follow-up EMBs according to the present guidelines.[22] The study protocol was set up in accordance to the ethics principles in the Declaration of

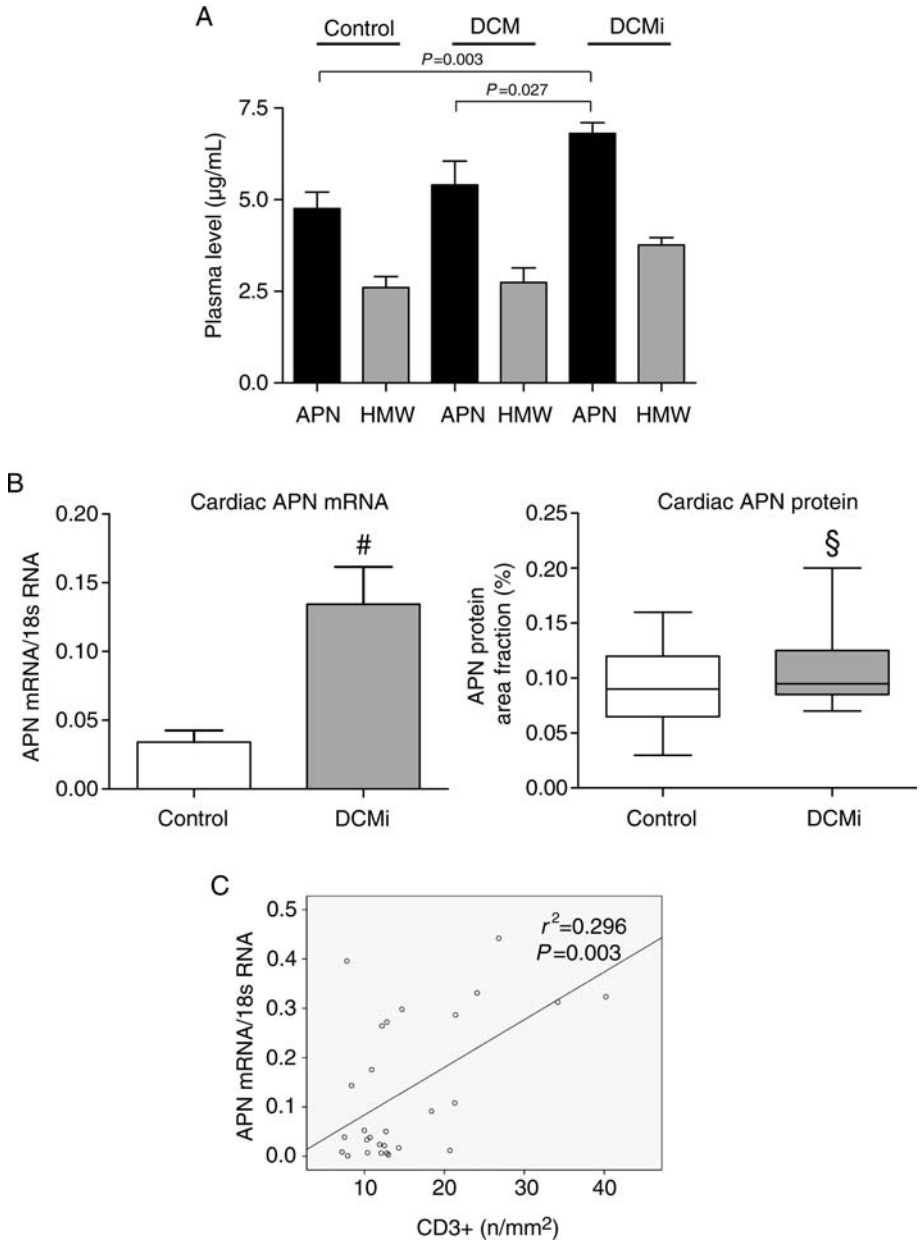


Figure 1 Adiponectin (APN) expression is elevated in human inflammatory cardiomyopathy (DCMi). (*A*) Plasma concentrations of APN and high molecular weight in the control ($n = 30$), DCM ($n = 30$), and DCMi ($n = 173$) groups were measured by ELISA. Data are presented as mean ± SEM. (*B*) Cardiac APN mRNA expression and protein expression were determined by QRT–PCR and immunohistochemistry, respectively. Significant differences are marked ($^{\#}P = 0.011$; $^{\S}P = 0.034$). (*C*) Correlation of cardiac APN mRNA expression with CD3+ positive cell number in patients with DCMi.

Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all patients.

Haemodynamic measurements and endomyocardial biopsies

Haemodynamic parameters of each patient were measured as previously described.[21] In brief, function of the left ventricle was studied by using the AVD system (Angiographic Ventricular Dynamics™) and by analysing the mechanical function of the heart by means of X-ray visualization during contrast injection. The 2-frame routine was used for calculation of LVEF. Left ventricular end-diastolic pressure (LVEDP) and capillary pressure (PC) were determined by catheterization with a tip catheter and cardiac index (CI) with a flow-directed catheter. In addition, echocardiography was performed on all patients to evaluate LVEDD. At least five EMBs were taken from all patients using a Cordis™ bioptom. Biopsies were analysed histologically and immunohistologically. Viral genomes were detected by polymerase chain reaction as described previously.[21]

Table 2 Correlations of haemodynamic parameters with adiponectin expression in inflammatory cardiomyopathy ($n = 173$)

	EF	LVEDD	LVEDP	PAP	PC	CI
APN	0.114 ($P < 0.001$)	0.043 ($P = 0.011$)	0.01 ($P = 0.248$)	0.13 ($P = 0.004$)	0.096 ($P = 0.005$)	0.266 ($P < 0.001$)
HMW	0.114 ($P < 0.001$)	0.028 ($P = 0.039$)	0.004 ($P = 0.447$)	0.166 ($P = 0.001$)	0.081 ($P = 0.01$)	0.226 ($P < 0.001$)

Data of correlation are presented as r^2. MMW and LMW did not correlate with haemodynamic parameters (data not shown).

Table 3 Correlation of adiponectin expression with inflammatory markers in patients with inflammatory cardiomyopathy ($n = 173$)

	CD3+	CD45 RO	IL-8	C-reactive protein	sTNF-R1	LFA1	Mac1
APN	0.025 ($P = 0.038$)	0.058 ($P = 0.018$)	0.106 ($P = 0.009$)	0.145 ($P = 0.002$)	0.14 ($P = 0.003$)	0.081 ($P = 0.016$)	0.056 ($P = 0.046$)
HMW	0.024 ($P = 0.042$)	0.079 ($P = 0.005$)	0.111 ($P = 0.007$)	0.132 ($P = 0.004$)	0.184 ($P = 0.001$)	0.054 ($P = 0.051$)	0.042 ($P = 0.041$)

Correlations are presented as r^2. HLA1, ICAM, VCAM, IL-2, IL-4, and IL-6 did correlate with plasma APN (data not shown).

Histological and immunhistological studies

Formalin fixed and paraffin embedded EMBs were assessed for inflammation and histological characteristics by light microscopy. Immunhistochemical analysis of inflammation [i.e. mononuclear infiltration (CD3+, MAC-1, LFA, CD45R0) as well as endothelial activation (ICAM, VCAM, HLA expression)] and cardiac APN expression were performed as previously described.[2,23,24]

Measurement of adiponectin and inflammatory cytokines

To assess total APN, HMW-APN, MMW-APN, and LMW-APN in human plasma, a specific assay from Immundiagnostik AG (Bensheim, Germany) was used according to the protocol supplied by the manufacturer. This system was capable of quantifying total APN and its different multimers in a single ELISA as described previously.[25] Human soluble TNF-receptor 1 (sTNFR1) and C-reactive protein were measured by ELISA (R&D Systems). Interleukines 2, 4, 6, 8, and 10 were determined by cytometric bead array (BD Biosciences).

Animal model of autoimmune myocarditis

To induce EAM, female BALB/c wild-type mice were immunized with 200 μL/mouse of a 1:1 emulsion of PBS with 1 mg/mL of MyHC-α peptide in CFA at Days 0 and 7. Control mice were immunized with PBS/CFA only. Replication defective adenoviral vectors expressing mouse APN under the control of a CMV promoter (Ad-APN) or control vectors (Ad-RR5) were injected intravenously 1 week before immunization (Day −7). Mice were analysed on Day 21. Hearts were removed and snap frozen. RNA was extracted by homogenizing the tissue in Trizol. Mouse cytokines and cytokine receptor RNA arrays were purchased from SA-Biosciences (Frederick, MD, USA) and performed as suggested by the manufacturer. For the synthesis of cDNA, the RT2 PCR Array First Strand Synthesis Kit (SA-Biosciences) was used. Analysis of chemokine and receptor expression was carried out employing the RT2 Profiler PCR Array System (SA-Biosciences). For mouse APN measurements, R&D Systems APN ELISA was employed according to the protocol provided by the manufacturer. TAQ-Man PCR was performed with mouse APN (Mm00456425_m1) and mouse APN receptor 1/2 (Mm01291331_m1, Mm01184032_m1), and primer sets were purchased from Applied Biosystems. Evaluation was performed by the DDCt method.

Characterization of antigen-specific T cell response

Mononuclear cells were isolated from healthy HLA-A2-positive volunteers by Ficoll-Hypaque density gradient centrifugation and were cryopreserved until analysis. For T cell expansion, mononuclear cells were incubated for 12 h without or with APN (3 μg/mL) and transferred into 96-well round-bottom plate (2×10^5 cells per well) together with influenza or Epstein-Barr virus (EBV) peptides or HIV peptides, rhIL-2 (50 IU/mL, R&D Systems), and rhIL-7 (10 ng/mL, R&D Systems) after APN incubation. Media and IL-2 were changed on Days 3 and 5. IL-7 was added after 1 week. After 10 days of culture, cells were harvested, washed, and evaluated for specific T cells by flow cytometry. Antigen-specific T cells were detected by intracellular cytokine staining after 6 h incubation with either an HLA-A2-binding influenza peptide or irrelevant HIV peptides as described previously.[26] In brief, peripheral blood mononuclear cells (PBMC) (2×10^6) were incubated with 10 μg/mL of each peptide. After 1 h, 7.5 μg/mL brefeldin A (Sigma, Deisenhofen, Germany) was added and after another 5 h, PBMC were surface-stained with fluorescence-conjugated monoclonal antibodies against CD3 and CD8 and intracellularly against IFN-γ, IL-2, and TNFα following fixation and permeabilization (BD Biosciences). Data acquisition was performed on a FACS Canto cytometer and analysed using DIVA Software (BD Bioscience).

NFκB-activation in cardiac myocytes and fibroblasts

Neonatal rat ventricular myocytes (NRVM) and fibroblasts were isolated and cultured as described previously.[2] Cells were then stimulated with TNFα (20 ng/mL) in the presence or absence of APN (10 μg/mL)

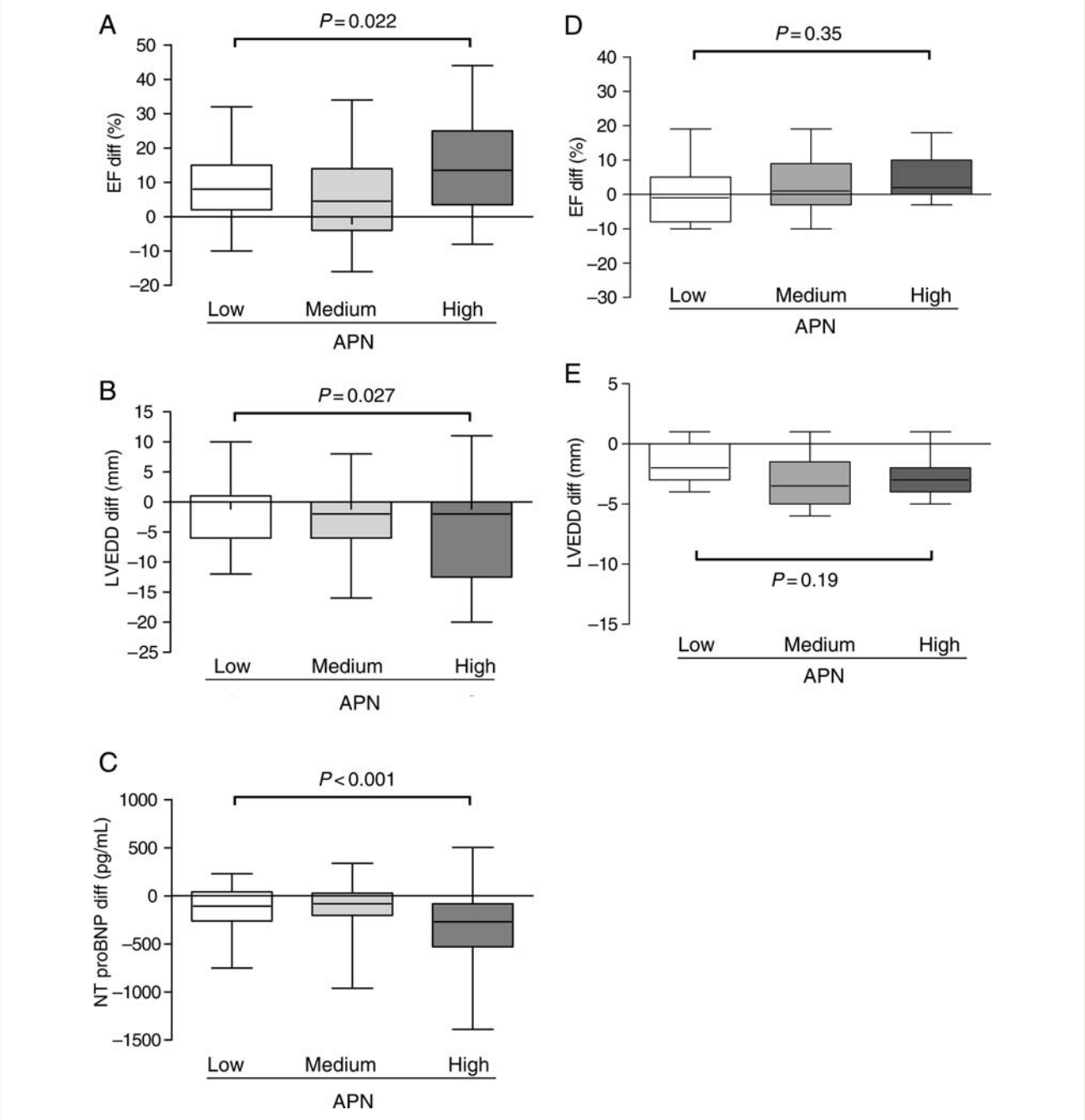


Figure 2 High adiponectin (APN) expression indicates favourable outcome at follow-up. (*A*) Inflammatory cardiomyopathy (DCMi) patients were separated into tertiles depending on their APN plasma concentrations at enrolment. Outcome is expressed as difference of ejection fraction (EF at follow up − EF at enrolment) in the following groups: (1) low adiponectin ($n = 58$), (2) medium adiponectin ($n = 58$), (3) high adiponectin ($n = 57$). (*B*) Outcome is expressed as difference in LVEDD (LVEDD at follow up − LVEDD at enrolment). (*C*) Outcome is expressed as difference in NT-proBNP (NT-proBNP at follow-up − NT-proBNP at enrolment). All data are presented in a box and whiskers appearance (median, 25% percentile, 75% percentile, ± SD). (*D* and *E*) DCM patients were separated into tertiles ($n = 10$) depending on their APN serum concentrations at enrolment. Outcome is expressed as difference of ejection fraction (EF at follow up − EF at enrolment) and as difference in left ventricular end-diastolic diameter (LVEDD) (LVEDD at follow up − LVEDD at enrolment).

or an NFκB inhibitor (BAY-11-7082, 20 μM, Sigma) for 30 min. Whole cells were lysed in sample buffer supplied and NFκB activation was determined by ELISA as described by the manufacturer (ActiveMotif®, Rixensart, Belgium).

Measurement of intracellular redox stress

Intracellular generation of reactive oxygen species (ROS) was determined using 2′,7′-dichlorofluorescin diacetate (DCFH-DA), which is rapidly oxidized to the highly fluorescent 2′,7′-dichlorofluorescin

Table 4 Follow-up of inflammatory cardiomyopathy patients—haemodynamic and inflammatory markers are shown for tertiles of adiponectin expression (CD3 diff rel: [CD3 diff/CD3 enrolment] ×100)

	1, $n = 58$	2, $n = 58$	3, $n = 57$
APN (μg/mL)	3.4 ± 0.74	5.8 ± 0.9	11.3 ± 3.3
EF1 (%)	35 ± 14	36 ± 12	26 ± 13
EF2 (%)	45 ± 15	43 ± 13	40 ± 16
EF diff (%)	9 ± 9	6 ± 12	15 ± 13
EF diff rel (%)	35.2 ± 49.5	21.8 ± 41.8	82.7 ± 94.1
LVEDD1 (mm)	60 ± 9.5	60 ± 10.4	65.2 ± 9.3
LVEDD2 (mm)	58 ± 9.3	56 ± 9.4	60 ± 10.0
LVEDD diff (mm)	−2.3 ± 5	−3.1 ± 5.3	−5.4 ± 7.1
LVEDD diff rel (%)	−3.7 ± 8.1	−4.8 ± 9.4	−8 ± 10.9
CD3 diff (n/mm^2)	−5.7 ± 3.5	−8.3 ± 7.9	−12.4 ± 13.9
CD3 diff rel (%)	−46.4 ± 22.3	−47.8 ± 29.4	−68.8 ± 15.3
BNP 1 (pg/mL)	576 ± 327	585 ± 360	906 ± 537
BNP 2 (pg/mL)	454 ± 282	444 ± 282	582 ± 437
BNP diff (pg/mL)	−122 ± 215	−138 ± 279	−356 ± 402

Data are presents as mean ± SD. The number of patients in each group for the values of CD3 is $n = 21$.

(DCF) in response to ROS production within the cells. Neonatal rat ventricular myocytes were cultured for 24 h in DMEM+ 10% FBS in 96 wells. Cells were then incubated in DMEM+ 1% FBS with or without APN (10 μg/mL) for 4 h. After washing in HBSS, cells were cultured in HBSS and DCFH-DA (DCFH-DA, Sigma) (8.64 mM) and H_2O_2 [400 mM, (Merck)] for 120 min. Fluorescence intensity was quantified every 10 min employing a fluorescence reader (excitation 480 nm, emission 540 nm) at 37°C (Molecular Devices).

Statistical analysis

For statistical analysis, SPSS statistical software version 16.0 was used. All data were expressed as mean ± SD. A value of $P < 0.05$ was regarded as statistically significant. A *t*-test for parametrically distributed data and a Wilcoxon matched-pair signed rank test for non-parametrically distributed data were utilized to compare parameters at the beginning and at the end of the study. Quantitative variables with normal distribution were analysed with student's *t*-test, and variables without normal distribution with a two-tailed Mann–Whitney *U*-test. Correlation analysis was performed with Pearson's and Spearman's test. Correction for Bonferroni was not performed. Multivariate linear regression was calculated to analyse the factors independently affecting specific variants. An α-error <5% was considered statistically significant. Factors such as CD3+ baseline cell numbers, APN baseline levels, LVEF, BMI, sex, age, C-reactive protein, brain natriuretic peptide (NT-proBNP), sTNFR1, and LVEDD were included in our analysis. Non-significant variants were subsequently excluded.

Results

Adiponectin concentrations are elevated in patients with inflammatory cardiomyopathy

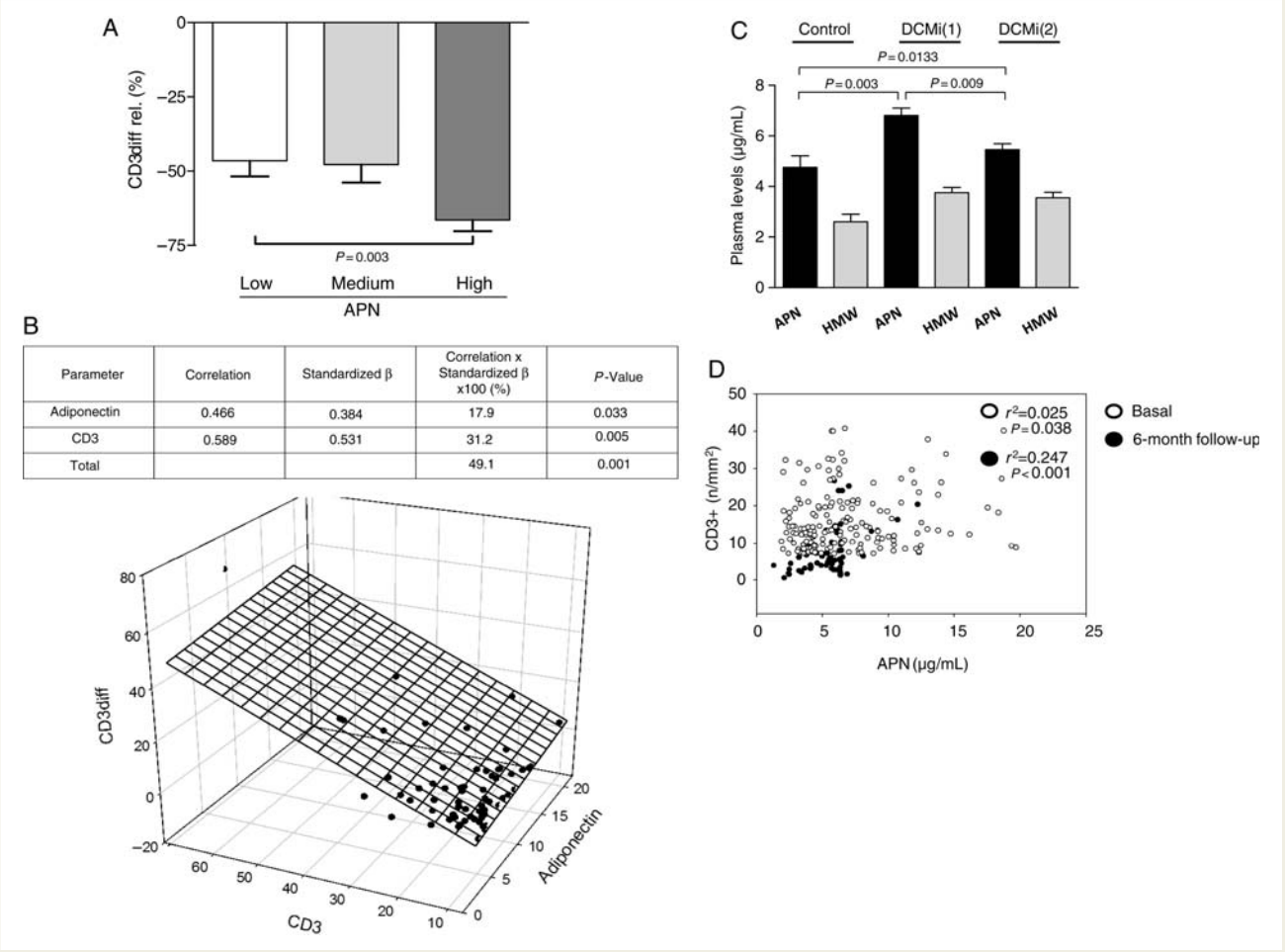
Patient characteristics for the study groups are shown in *Table 1*. As illustrated in *Figure 1A*, systemic APN concentrations in patients with DCMi (median: 5.73 μg/mL, 25% percentile: 4.06 μg/mL, 75% percentile: 8.84 μg/mL) were significantly higher when compared with DCM patients (median: 4.67 μg/mL, 25% percentile: 2.99 μg/mL, 75% percentile: 6.38 μg/mL) and controls (median: 4.52 μg/mL, 25% percentile: 2.68 μg/mL, 75% percentile: 5.97 μg/mL). High molecular weight APN concentrations also differed significantly. Moreover, cardiac APN mRNA expression in DCMi (APNmRNA/18sRNA: 0.034 ± 0.01 vs. 0.135 ± 0.03, $P = 0.011$) and protein expression in DCMi (area fraction: 0.089 ± 0.04 vs. 0.12 ± 0.01, $P = 0.034$) were significantly upregulated (*Figure 1B*).

Adiponectin concentrations in patients with inflammatory cardiomyopathy correlate with cardiac haemodynamics and inflammation

In order to further investigate factors associated with elevated APN plasma levels in patients with DCMi, haemodynamics and the amount of cardiac mononuclear inflammatory infiltrates were analysed. Plasma APN concentrations were inversely correlated with LVEF. A similar pattern was observed for HMW-APN. An overview about correlations of APN levels with invasively measured haemodynamic parameters is depicted in *Table 2*. Furthermore, APN plasma concentrations correlated in a significant manner with CD3+ T cell and CD45R0+ memory T cell number in EMB specimens at time of inclusion into the study (*Table 3*), while no correlation could be demonstrated between EF and mononuclear infiltration ($r^2 = 0.004$, $P = 0.433$). Those data indicate a relationship between impairment of cardiac function as well as the extent of cardiac inflammation with systemic APN concentrations. Furthermore, plasma concentrations of IL-8, soluble sTNFR1, and C-reactive protein significantly correlated with APN plasma levels, implicating the inflammatory process in the regulation of APN (*Table 3*). In support of this contention, cardiac APN mRNA and protein expression were upregulated in DCMi and significantly correlated with cardiac inflammation (*Figure 1B* and *C*).

High adiponectin serum concentrations indicate better outcome and reduced cardiac inflammation at follow-up in inflammatory cardiomyopathy patients

Patients were evaluated at 6 months follow-up and divided into three groups according to their baseline APN plasma concentrations. As illustrated in *Figure 2A* and *Table 4*, patients with high APN plasma levels at baseline exhibited a better improvement in LVEF compared with patients in the lowest tertile of baseline APN concentrations. Accordingly, LVEDD significantly improved in patients in the upper tertile of serum APN compared with patients with lower APN baseline levels (*Figure 2B*). In accordance with those functional parameters, NT-proBNP, a marker for prognosis in DCMi, showed a significant improvement at follow-up in high APN expressing patients (NT-proBNP: $P<0.001$; *Figure 2C*). In contrast, APN effects on outcome were not observed in DCM patients (*Figure 2D* and *E*). These data suggest that APN expression is associated with favourable outcome in DCMi patients, and might implicate a protective effect of the adipocytokine. In fact, analysis of follow-up heart biopsy specimens ($n = 63$) revealed an association of high baseline



Parameter	Correlation	Standardized β	Correlation x Standardized β x100 (%)	P-Value
Adiponectin	0.466	0.384	17.9	0.033
CD3	0.589	0.531	31.2	0.005
Total			49.1	0.001

Figure 3 High APN expression is associated with diminished cardiac inflammation in the course of inflammatory cardiomyopathy (DCMi). (*A*) CD3+ T cells were determined in endomyocardial biopsies by immunohistological staining at enrolment and six month follow up. 63 consecutive DCMi patients were grouped according to their APN serum concentrations ($n = 21$ in each group). These 3 groups were compared according to CD3+ cells at baseline and follow-up. Bar graph illustrates relative improvement within these groups. (*B*) Multivariate linear regression model for CD3diff (CD3diff: [CD3+ cells at follow-up] − [CD3+ cells at baseline]). Linear regression was calculated after inclusion of potential co-variants. Non-significant co-variants were subsequently excluded, leaving APN and CD3+ as contributors within the most efficient model. The multiplicative term (correlation x standardized β ×100) explains the variation of CD3diff illustrated by the respective parameter in percent. (*C*) APN plasma concentrations in DCMi patients at inclusion into the study (DCMi 1) and at 6 month follow-up (DCMi 2). Values are expressed as Mean ± SD. (*D*) APN and cardiac mononuclear infiltration at inclusion and at 6 month follow-up. APN concentrations were measured by ELISA, CD3+ cells were determined by immunohistological staining.

APN levels with lower numbers of heart infiltrating cells at 6 months. As shown in *Figure 3A*, the decrease of CD3+ T cells was most pronounced in patients with high serum APN concentrations (high vs. low APN: 67 ± 17 vs. 47 ± 23%, $P = 0.003$). In order to address the question whether APN is an independent prognostic factor for inflammation in DCMi, a multivariate linear regression analysis was performed, including CD3+ baseline cell numbers, APN baseline levels, age ($P = 0.76$), NT-proBNP ($P = 0.78$), sTNR-R1 ($P = 0.83$), sex ($P = 0.63$), BMI ($P = 0.53$), C-reactive protein ($P = 0.54$), LVEF ($P = 0.25$), and LVEDD ($P = 0.45$). Interestingly both CD3+ baseline cell numbers and APN baseline levels had a considerable impact on the CD3 changes at 6-month follow-up (CD3diff): as shown in *Figure 3B* and *Table 4*, higher systemic APN concentrations and CD3 cell counts at study entry were associated with higher CD3diff changes on follow-up. Both parameters alone accounted for ∼49% of the variation of CD3diff. Moreover, in line with lower myocardial inflammation, APN levels were decreased compared with study inclusion, but still elevated compared with controls and still correlated with cardiac inflammation (*Figure 3C* and *D*).

Murine autoimmune myocarditis is associated with high adiponectin expression and adiponectin gene transfer inhibits cardiac inflammation

To further test our hypothesis that APN acts as an anti-inflammatory cytokine in inflammatory cardiomyopathy and to corroborate our human data, we used an EAM model, which

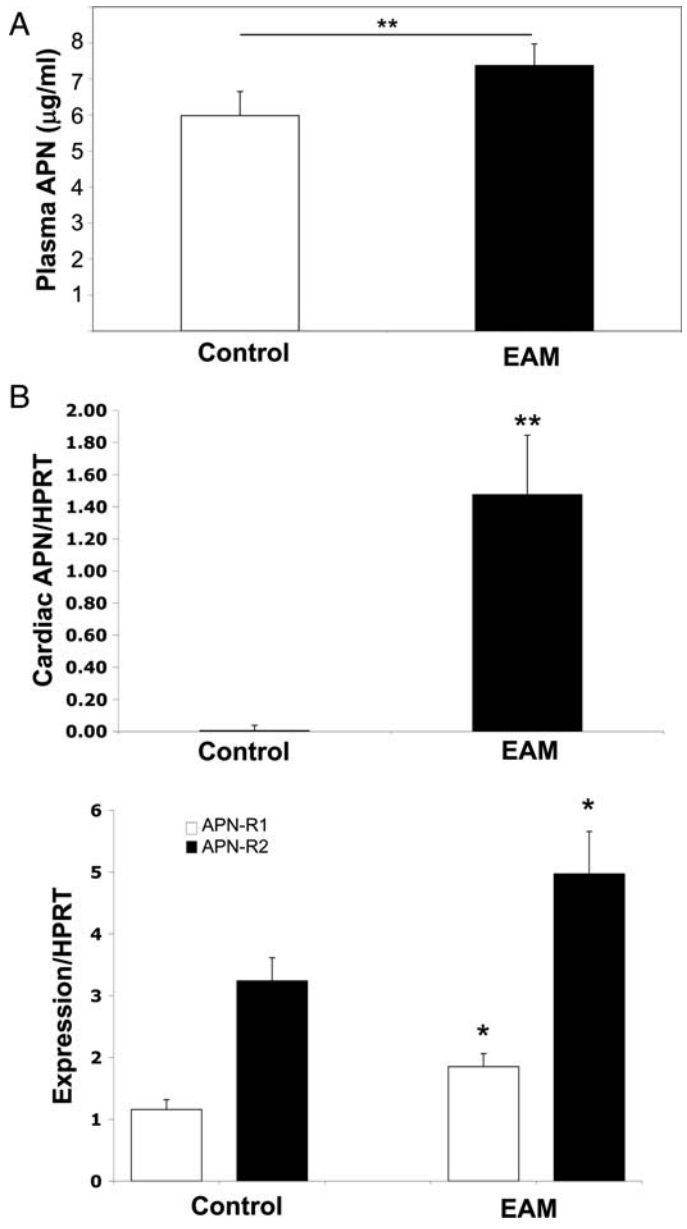


Figure 4 Adiponectin (APN) gene transfer results in downregulation of multiple inflammatory mediators upregulated in murine autoimmune myocarditis. (*A*) APN plasma concentration in mice with experimental autoimmune myocarditis (EAM). Three weeks after induction of EAM, blood was drawn and APN concentrations were determined. Values are presented as mean ± SD ($n = 6$ in each group), $^{*}P < 0.05$ control vs. EAM. (*B*) Cardiac APN signalling is increased in EAM. mRNA expression of APN and its receptors (APN receptor 1/2) were determined in hearts of mice 3 weeks after EAM expression. Values are mean ± SD ($n = 6$ animals in each group, $^{*}P < 0.05$, $^{**}P < 0.01$ control vs. EAM). (*C*) Mouse chemokine and -receptors PCR array depicting change in expression of 84 genes in EAM. At 3 weeks following immunization, RNA was extracted from hearts and gene expression measured. Thirty-two genes were upregulated and none was downregulated in EAM. APN gene transfer caused a downregulation of 22 genes. 3D profiles and scatter blots are depicted for experimental groups as indicated. (*D*) APN gene transfer significantly reduces cardiac levels of chemokines and its receptors involved in the pathogenesis of murine autoimmune myocarditis. (*E*) APN gene transfer inhibits cardiac upregulation of pro-inflammatory cytokines in EAM determined by QRT–PCR.

mirrors mechanistic aspects of human DCMi in mice. Groups of mice were immunized with either a heart-specific alpha myosin heavy chain peptide together with the adjuvant CFA or with CFA only. In line with the human data, APN plasma concentrations were significantly higher in mice with EAM, compared with controls immunized with CFA only (5.9 ± 0.6 vs. 7.4 ± 0.6 µg/mL, $P<0.01$, $n = 6$). Furthermore, cardiac expression of APN as well as APN receptor 1/2 was significantly increased in EAM mice (*Figure 4A* and *B*), demonstrating enhanced APN signalling. Those data implicate the inflammatory process in the regulation

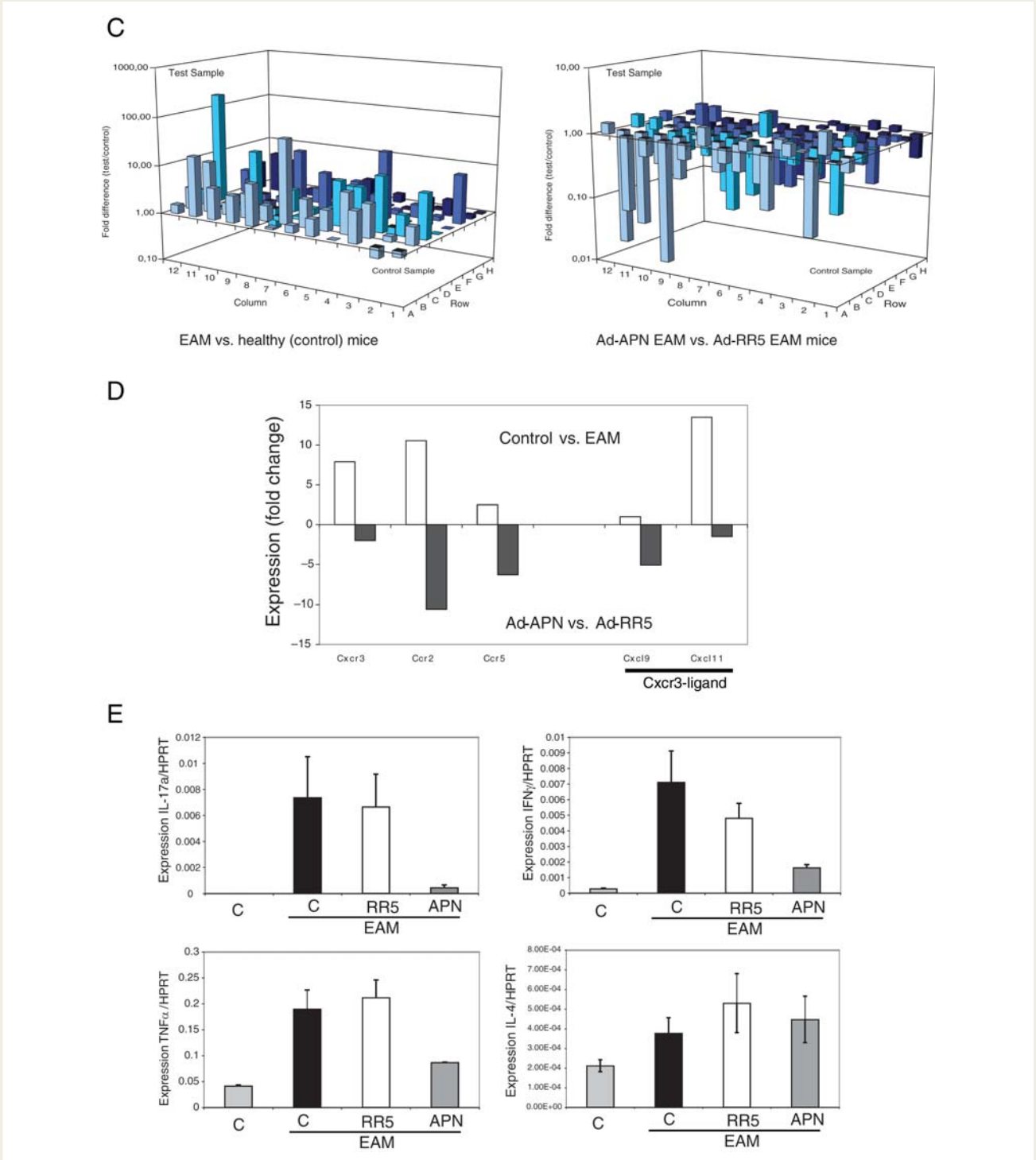


Figure 4 (Continued)

of APN expression. To test the hypothesis that APN acts as an endogenous anti-inflammatory protein, groups of mice were either transduced with an adenoviral vector, expressing mouse APN, or a vehicle before immunization with CFA/MyHC-α. Three weeks later, heart tissue was analysed for cytokine and chemokine expression using PCR array. Experimental autoimmune myocarditis induction was associated with significant upregulation of multiple inflammatory markers. Several cytokines, reflecting active cardiac inflammation, such as TNFα, IFNγ, IL-4, and IL-17 as well as chemokines, and their receptors were strongly upregulated (*Figure 4C–E*). Remarkably, APN gene transfer caused downregulation of multiple chemokines and cytokines, including TNFα and IL-17, well known to play a central role in the persistence and progression of EAM (*Figure 4E*). In line with the observed

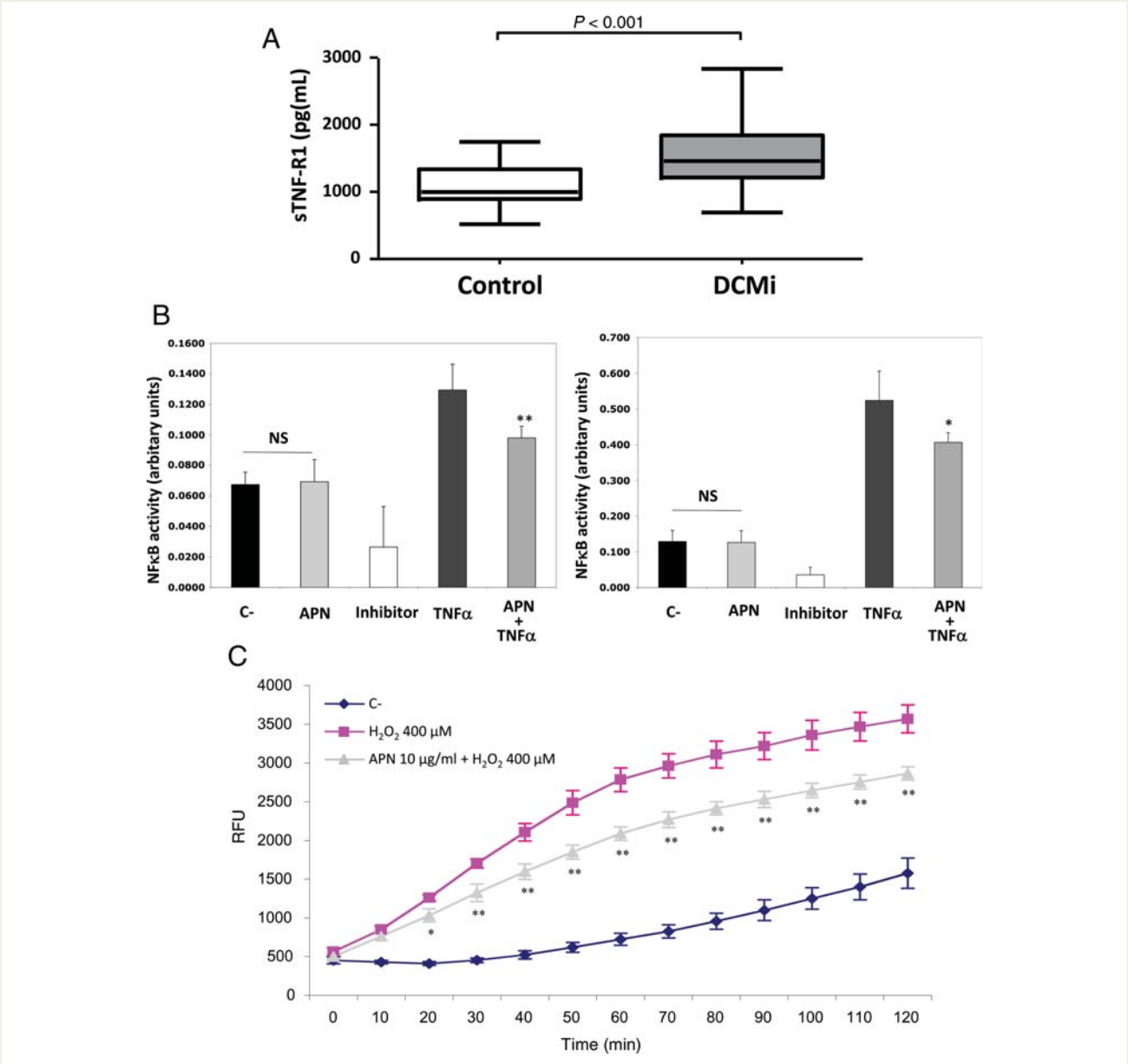


Figure 5 Adiponectin (APN) inhibits the inflammatory response in cardiac cells *in vitro*. (*A*) Soluble TNF-receptor 1 (sTNFR1) was measured by ELISA in plasma samples of patients. Box plot illustrates elevated sTNFR1 in inflammatory cardiomyopathy ($n = 173$) indicating augmented TNFα-signalling compared with control ($n = 30$). (*B*) APN inhibits TNFα-induced NFκB activation *in vitro*: neonatal rat ventricular myocytes (NRVM) (left) and neonatal rat fibroblasts (right) were isolated, cultured, and incubated with TNFα for 30 min in the presence or absence of APN and an NFκB inhibitor, BAY-11-7082 (20 μM). Cells were then harvested and whole cell lysates were used for the determination of NFκB activity by ELISA. Values are expressed as mean ± SD for three independent experiments performed in triplicates (*$P < 0.05$, **$P < 0.01$). (*C*) APN inhibits production of reactive oxygen species: NRVM were isolated and incubated with H2O2. Fluorescence intensity was measured (RFU, relative fluorescence intensity). Data for different time points are expressed as mean ± SD for three independent experiments performed in triplicate (*$P < 0.05$, **$P < 0.01$).

downregulation of several inflammatory key cytokines, APN mice had up to five-fold lower expression of the type 1 chemokine receptor CXCR3 and its ligands CXCL9/CXCL11 (*Figure 4D*). Interestingly, strong downregulation of both CCR2 and CCR5, which have been shown to be crucial in EAM,[27] was also observed. Taken together, these data indicate a potent anti-inflammatory effect of APN in inflammatory heart disease.

Adiponectin inhibits inflammation in cardiomyocytes *in vitro*

Adiponectin has been shown to possess immunomodulating and anti-inflammatory effects. Inflammatory cardiomyopathy is associated with increased TNFα signalling via its downstream target NFκB. Similarly, TNF-alpha signalling is critically required for the

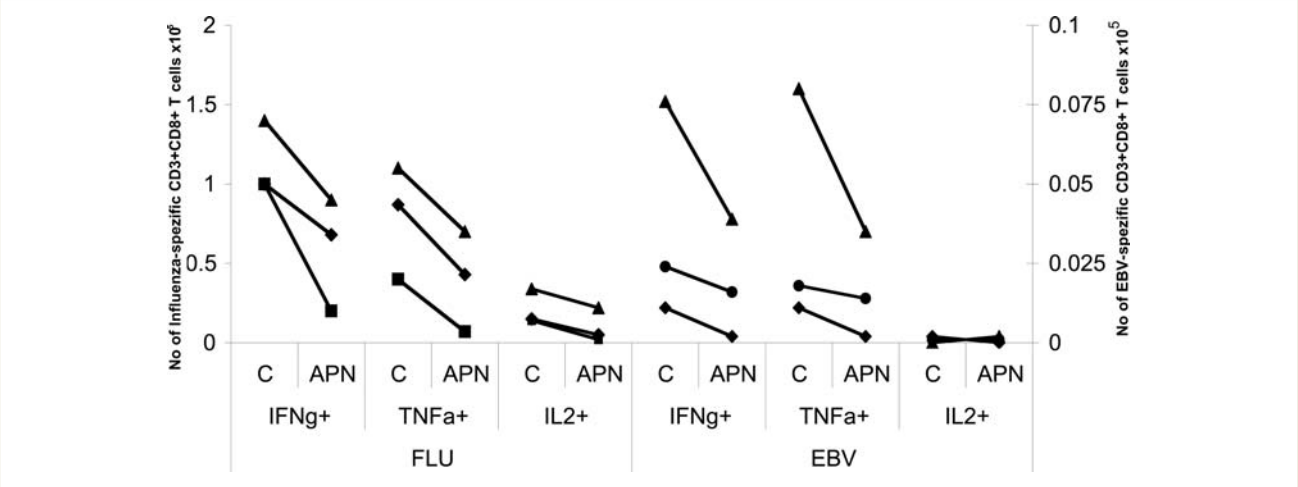


Figure 6 Adiponectin (APN) inhibits expansion of antigen-specific T cells. T cells in peripheral blood mononuclear cell samples were stimulated with influenza or EBV or irrelevant HIV peptides in the presence of IL-2 and IL-7 with or without APN. At Day 10, numbers of antigen-specific T cells were determined by cytokine production by flow cytometry. Six independent experiments using different donors are shown ($P < 0.05$ for EBV, $P < 0.01$ for Influenza responses, respectively).

induction and progression of EAM.[28] In our study population, patients with DCMi exhibited significantly elevated sTNFR-1 serum concentrations (DCMi vs. control: 1533 ± 448 vs. 1098 ± 321 pg/mL, $P < 0.001$), a marker for activated TNFα signalling (*Figure 5A*). In order to further elucidate the effects of APN on TNFα signal transduction within the myocardium, we performed *in vitro* cell culture experiments. Following TNFα stimulation, APN inhibited the activation of NFκB, a master transcription factor activated in inflammation, in NRVM as well as in neonatal rat fibroblasts *in vitro* ($P < 0.05$, *Figure 5B*). These data implicate an anti-inflammatory effect of APN. Increased redox stress has been implicated in DCMi progression and heart failure. Accordingly, we analysed the effect of APN on the production of ROS in cardiac myocytes. Production of ROS was significantly diminished in cardiac myocytes following H_2O_2 incubation in the presence of APN ($P < 0.01$) (*Figure 5C*). Taken together, our data implicate that inhibition of NFκB activation by APN as well as a reduction of ROS generation as possible protective mechanisms in DCMi patients.

Adiponectin inhibits antigen specific T cell responses *in vitro*

Adiponectin inhibits monocyte function *in vitro* including cytokine expression and phagocytosis. However, very little is known about the effect of APN on T cells, which play a critical role in DCMi progression. In fact, mononuclear infiltrates in myocardial tissue have been recently shown to be an independent positive predictor for clinical outcome of patients with inflammatory cardiomyopathy.[11] In our study, high baseline APN expression was associated with lower CD3 infiltrates at follow-up. To investigate a potential direct effect of APN on T cell expansion, we therefore analysed the influence of APN on the generation of antigen-specific T cell responses *in vitro*. Since it is difficult to raise specific human T cell lines against myocardial antigens, the influence of APN on the generation of influenza and EBV-specific T cells was analysed. PBMCs were stimulated with antigens and expanded *in vitro* for 10 days. Adiponectin pre-incubation resulted in significant lower numbers of influenza-specific ($P < 0.01$) as well as EBV-specific ($P < 0.05$) IFNγ, TNFα, and IL-2 producing T cells (*Figure 6*). Taken together, these data indicate that APN inhibits expansion of antigen specific T cells and might therefore be involved in a negative feedback mechanism confining overhelming T cell responses and chronic inflammation.

Discussion

This study elucidated the effects of APN in inflammatory cardiomyopathy. We demonstrate, for the first time, that enhanced APN expression parallels cardiac and systemic inflammation in patients with DCMi. However, patients with high APN showed decreased inflammatory cell infiltrates in their myocardium, improved haemodynamics, and decrease NT-proBNP levels on follow-up when compared with those with low APN levels. These data suggest a regulatory role of APN in DCMi. Mice with EAM also exhibited increased APN expression at the peak of inflammation, and APN overexpression significantly inhibited the upregulation of key chemokines/chemokine receptors and pro-inflammatory cytokines known to mediate disease progression in this model of human DCMi. *In vitro*, APN inhibited NFκB-signalling in cardiomyocytes as well as expansion of antigen-specific human T cells. Our data therefore implicate a novel function of APN as anti-inflammatory cytokine confining cardiac inflammation and disease progression in DCMi.

Human DCMi is characterized by mononuclear myocardial inflammatory infiltrates and commonly results from infections with cardiotropic viruses.[29,30] Recent studies indicate that dilated cardiomyopathy develops as late sequelae of chronic myocarditis because of viral persistence or a chronic immune process initially

triggered by viral infection.[21,29] Chronic inflammation is frequently followed by heart-specific autoimmunity.[31,32] A breakdown in the control mechanisms protecting against autoimmune reactions by both presentation of normally not accessible self-antigens and bystander-activation, induced by the pathogen, leads to the formation of auto- reactive antibodies and T cells.[33,34] In this regard, a recent observational study by Kindermann *et al.*[11] demonstrated that immunohistological signs of inflammation rather than viral infection per se predict outcome of patients with myocarditis and cardiac dysfunction.[11] Thus, our current mechanistic concept emanates from the assumption that an initially beneficial cardiac immune response will become deleterious if control and final resolution of the immune response is disturbed.

Here we show that plasma APN correlates with systemic markers of inflammation (i.e., IL-8, sTNFR1, C-reactive protein) and plasma as well as cardiac APN with myocardial mononuclear infiltrates (i.e., CD3, CD45R0, Mac-1, LFA-1) in patients with DCMi at baseline. This is consistent with elevated plasma levels of the adipocytokine in other inflammatory states—such as lupus erythematosus, rheumatoid arthritis, or inflammatory bowel disease.[14] In contrast to downregulation of local cardiac APN expression in DCM,[2] cardiac expression of APN in patients with cardiac inflammation (DCMi) was significantly upregulated suggesting the inflammatory process in the regulation of APN expression. The mechanism for enhanced APN expression, however, remains elusive, but appears to be independent of TNFα signalling, known as a negative regulator of APN, since sTNFR1 was enhanced in our patient population. Data obtained in mouse EAM corroborate these findings, since cardiac infiltrates were associated with high plasma and cardiac APN. Therefore, it is tempting to speculate that the organ-specific inflammatory process by itself upregulates APN expression as suggested by upregulation of cardiac expression of APN and its receptors in EAM mice and DCMi patients.

Previous studies characterized APN as immunomodulatory bifunctional cytokine exerting both pro-inflammatory and anti-inflammatory effects. *In vitro*, the induction of proinflammatory cytokines by APN has mainly been studied in unstimulated cells, whereas the induction of anti-inflammatory cytokines and attenuation of adhesion and phagocytosis have mostly been observed in stimulated cells. Extrapolating to the *in vivo* situation, it is possible that in an inflammatory milieu, APN mitigates inflammation. In line with this, DCMi patients with high APN serum levels in our study exhibited a significant decrease in myocardial mononuclear infiltration at 6-month follow-up. Low cardiac infiltration was associated with favourable outcome, an observation not found in DCM patients. Thus, one may speculate that high APN levels, although initially associated with enhanced cardiac inflammation, may exert a long-term anti-inflammatory effect. In fact, our multivariate analysis revealed that APN explains ~18% of the variability of CD3 counts at follow-up. This hypothesis is supported by our *in vivo* data from the EAM model. In fact, APN gene transfer resulted in diminished RNA expression of various inflammatory molecules in mice with EAM. Most interestingly, IL-17 was found to be downregulated playing a central role in EAM progression by promoting recruitment of CD11b+ monocytes, the major heart-infiltrating cells.[35] In line with this finding, both CCR2 and CCR5, which were shown to mediate migration of CD11b+ monocytes in EAM, were diminished in APN vector transfected mice as well.[27] Importantly, Day 21 reflects the peak of inflammation in the EAM model. As expected, and in line with the observations in the patients at baseline, APN levels are elevated at this time point. Further long-term experiments including serial cardiac morphological and functional investigations will be required to comprehensively characterize all APN actions in the EAM model, i.e. effects on cardiomyopathy phenotype. Nevertheless, our data already strongly support the contention that endogenously released APN acts as protective cytokine downregulating the inflammatory process in DCMi. To further study the potential effect of APN on the antigen-specific T cell responses, we performed *in vitro* studies. In line with our concept, we could observe that APN exerts an inhibitory effect on the expansion of anti-viral memory and effector T cell responses *in vitro*.

Our findings are in accordance with previous studies of the role of APN in inflammatory models. Reduced APN expression in cyclooxygenase-2 deficient mice was associated with enhanced inflammatory cell damage in a model of EMC virus-induced myocarditis.[36] Moreover, inflammatory myocardial damage was attenuated in leptin-deficient mice with viral myocarditis following APN replacement therapy.[18] Furthermore, induction of cardiac APN expression by candesartan diminished cardiac damage in obese mice with viral myocarditis[37] and improved survival.[38] Taken together, our data complete other studies pointing to an anti-inflammatory and cardioprotective role of APN in inflammatory cardiomyopathy.

Our *in vitro* experiments also implicate direct anti-inflammatory effects of APN in cardiac myocytes and fibroblasts both showing diminished NFκB activation after TNFα stimulation. TNFα depresses cardiac contractility, induces cardiomyocyte apoptosis, activates the inflammatory response,[39] and induces dilated cardiomyopathy.[40,41] Therefore, TNFα, upregulated in patients with DCMi in our study as assessed by sTNFR1, might be a key mediator in the development of heart failure. NFκB is an important transcription factor mediating the detrimental and pro-inflammatory effects of TNFα, and abrogation of NFκB in TNFα transgenic mice improved survival and severity of DCM.[42] Therefore, inactivation of NFκB, a central transcription factor in driving the inflammatory and remodelling response downstream of TNFα, might diminish endomyocardial inflammation and reorganization of cell–matrix interactions. In fact, NFκB has direct effects on apoptosis, calcium handling, cell adhesion, and expression of inflammatory proteins (i.e., COX2, iNOS). Recently, NFκB induction has been demonstrated in the failing human heart.[43,44] Moreover, silencing of NFκB prevented heart failure and hypertrophy in mouse models of pressure overload as well as after coronary artery ligation.[45] In this regard, APN knockout mice showed higher mortality and larger LVEDD in a pressure overload model.[46] Taken together, APN might diminish the inflammatory response as well as left ventricular remodelling also by direct inhibition of NFκB activation in cardiac myocytes and fibroblasts.

In conclusion, our data point to a novel anti-inflammatory role of APN in human inflammatory heart disease and suggest to explore APN as a novel therapeutic concept.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

References

1. Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett* 2005;**579**:5163–5169.
2. Skurk C, Wittchen F, Suckau L, Witt H, Noutsias M, Fechner H, Schultheiss HP, Poller W. Description of a local cardiac adiponectin system and its deregulation in dilated cardiomyopathy. *Eur Heart J* 2008;**29**:1168–1180.
3. Bobbert T, Rochlitz H, Wegewitz U, Akpulat S, Mai K, Weickert MO, Mohlig M, Pfeiffer AF, Spranger J. Changes of adiponectin oligomer composition by moderate weight reduction. *Diabetes* 2005;**54**:2712–2719.
4. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, Kumada M, Sato K, Schiekofer S, Ohashi K, Funahashi T, Colucci WS, Walsh K. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004;**10**:1384–1389.
5. Shibata R, Izumiya Y, Sato K, Papanicolaou K, Kihara S, Colucci WS, Sam F, Ouchi N, Walsh K. Adiponectin protects against the development of systolic dysfunction following myocardial infarction. *J Mol Cell Cardiol* 2007;**42**:1065–1074.
6. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004;**291**:1730–1737.
7. George J, Patal S, Wexler D, Sharabi Y, Peleg E, Kamari Y, Grossman E, Sheps D, Keren G, Roth A. Circulating adiponectin concentrations in patients with congestive heart failure. *Heart* 2006;**92**:1420–1424.
8. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, Hildebrandt P. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation* 2005;**112**:1756–1762.
9. McEntegart MB, Awede B, Petrie MC, Sattar N, Dunn FG, MacFarlane NG, McMurray JJ. Increase in serum adiponectin concentration in patients with heart failure and cachexia: relationship with leptin, other cytokines, and B-type natriuretic peptide. *Eur Heart J* 2007;**28**:829–835.
10. Ingelsson E, Riserus U, Berne C, Frystyk J, Flyvbjerg A, Axelsson T, Lundmark P, Zethelius B. Adiponectin and risk of congestive heart failure. *JAMA* 2006;**295**:1772–1774.
11. Kindermann I, Kindermann M, Kandolf R, Klingel K, Bultmann B, Muller T, Lindinger A, Bohm M. Predictors of outcome in patients with suspected myocarditis. *Circulation* 2008;**118**:639–648.
12. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004;**323**:630–635.
13. Kim KY, Kim JK, Han SH, Lim JS, Kim KI, Cho DH, Lee MS, Lee JH, Yoon DY, Yoon SR, Chung JW, Choi I, Kim E, Yang Y. Adiponectin is a negative regulator of NK cell cytotoxicity. *J Immunol* 2006;**176**:5958–5964.
14. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. *J Allergy Clin Immunol* 2008;**121**:326–330.
15. Rovin BH, Song H. Chemokine induction by the adipocyte-derived cytokine adiponectin. *Clin Immunol* 2006;**120**:99–105.
16. Haugen F, Drevon CA. Activation of nuclear factor-kappaB by high molecular weight and globular adiponectin. *Endocrinology* 2007;**148**:5478–5486.
17. Takahashi T, Yu F, Saegusa S, Sumino H, Nakahashi T, Iwai K, Morimoto S, Kurabayashi M, Kanda T. Impaired expression of cardiac adiponectin in leptin-deficient mice with viral myocarditis. *Int Heart J* 2006;**47**:107–123.
18. Takahashi T, Saegusa S, Sumino H, Nakahashi T, Iwai K, Morimoto S, Kanda T. Adiponectin replacement therapy attenuates myocardial damage in leptin-deficient mice with viral myocarditis. *J Int Med Res* 2005;**33**:207–214.
19. Kanda T, Saegusa S, Takahashi T, Sumino H, Morimoto S, Nakahashi T, Iwai K, Matsumoto M. Reduced-energy diet improves survival of obese KKAy mice with viral myocarditis: induction of cardiac adiponectin expression. *Int J Cardiol* 2007;**119**:310–318.
20. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, Kihara S, Walsh K. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest* 2007;**117**:375–386.
21. Kuhl U, Pauschinger M, Noutsias M, Seeberg B, Bock T, Lassner D, Poller W, Kandolf R, Schultheiss HP. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with "idiopathic" left ventricular dysfunction. *Circulation* 2005;**111**:887–893.
22. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, Levine GN, Narula J, Starling RC, Towbin J, Virmani R. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation* 2007;**116**:2216–2233.
23. Noutsias M, Seeberg B, Schultheiss HP, Kuhl U. Expression of cell adhesion molecules in dilated cardiomyopathy: evidence for endothelial activation in inflammatory cardiomyopathy. *Circulation* 1999;**99**:2124–2131.
24. Warraich RS, Noutsias M, Kazak I, Seeberg B, Dunn MJ, Schultheiss HP, Yacoub MH, Kuhl U. Immunoglobulin G3 cardiac myosin autoantibodies correlate with left ventricular dysfunction in patients with dilated cardiomyopathy: immunoglobulin G3 and clinical correlates. *Am Heart J* 2002;**143**:1076–1084.
25. Bobbert P, Rauch U, Stratmann B, Goldin-Lang P, Antoniak S, Bobbert T, Schultheiss HP, Tschoepe D. High molecular weight adiponectin correlates positively with myeloperoxidase in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2008;**82**:179–184.
26. Scheibenbogen C, Letsch A, Thiel E, Schmittel A, Mailaender V, Baerwolf S, Nagorsen D, Keilholz U. CD8 T-cell responses to Wilms tumor gene product WT1 and proteinase 3 in patients with acute myeloid leukemia. *Blood* 2002;**100**:2132–2137.
27. Goser S, Ottl R, Brodner A, Dengler TJ, Torzewski J, Egashira K, Rose NR, Katus HA, Kaya Z. Critical role for monocyte chemoattractant protein-1 and macrophage inflammatory protein-1alpha in induction of experimental autoimmune myocarditis and effective anti-monocyte chemoattractant protein-1 gene therapy. *Circulation* 2005;**112**:3400–3407.
28. Bachmaier K, Pummerer C, Kozieradzki I, Pfeffer K, Mak TW, Neu N, Penninger JM. Low-molecular-weight tumor necrosis factor receptor p55 controls induction of autoimmune heart disease. *Circulation* 1997;**95**:655–661.
29. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006;**113**:1807–1816.
30. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, Martin I, Nordet P. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996;**93**:841–842.
31. Caforio AL, Calabrese F, Angelini A, Tona F, Vinci A, Bottaro S, Ramondo A, Carturan E, Iliceto S, Thiene G, Daliento L. A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis. *Eur Heart J* 2007;**28**:1326–1333.
32. Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. *Circulation* 2003;**107**:857–863.
33. Eriksson U, Ricci R, Hunziker L, Kurrer MO, Oudit GY, Watts TH, Sonderegger I, Bachmaier K, Kopf M, Penninger JM. Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat Med* 2003;**9**:1484–1490.
34. Rose NR. Infection, mimics, and autoimmune disease. *J Clin Invest* 2001;**107**:943–944.
35. Valaperti A, Marty RR, Kania G, Germano D, Mauermann N, Dirnhofer S, Leimenstoll B, Blyszczuk P, Dong C, Mueller C, Hunziker L, Eriksson U. CD11b+ monocytes abrogate Th17 CD4+ T cell-mediated experimental autoimmune myocarditis. *J Immunol* 2008;**180**:2686–2695.
36. Takahashi T, Zhu SJ, Sumino H, Saegusa S, Nakahashi T, Iwai K, Morimoto S, Kanda T. Inhibition of cyclooxygenase-2 enhances myocardial damage in a mouse model of viral myocarditis. *Life Sci* 2005;**78**:195–204.
37. Yu F, Chen R, Takahashi T, Sumino H, Morimoto S, Nakahashi T, Iwai K, Matsumoto M, Kanda T. Candesartan improves myocardial damage in obese mice with viral myocarditis and induces cardiac adiponectin. *Int J Cardiol* 2008;**129**:414–421.
38. Saegusa S, Fei Y, Takahashi T, Sumino H, Moriya J, Kawaura K, Yamakawa J, Itoh T, Morimoto S, Nakahashi T, Iwai K, Matsumoto M, Kanda T. Oral administration of candesartan improves the survival of mice with viral myocarditis through modification of cardiac adiponectin expression. *Cardiovasc Drugs Ther* 2007;**21**:155–160.

39. Tang Z, McGowan BS, Huber SA, McTiernan CF, Addya S, Surrey S, Kubota T, Fortina P, Higuchi Y, Diamond MA, Wyre DS, Feldman AM. Gene expression profiling during the transition to failure in TNF-alpha over-expressing mice demonstrates the development of autoimmune myocarditis. *J Mol Cell Cardiol* 2004;**36**:515–530.
40. Kubota T, McTiernan CF, Frye CS, Demetris AJ, Feldman AM. Cardiac-specific overexpression of tumor necrosis factor-alpha causes lethal myocarditis in transgenic mice. *J Card Fail* 1997;**3**:117–124.
41. Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, Mann DL. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. *Circulation* 2001;**104**:826–831.
42. Higuchi Y, Chan TO, Brown MA, Zhang J, DeGeorge BR Jr., Funakoshi H, Gibson G, McTiernan CF, Kubota T, Jones WK, Feldman AM. Cardioprotection afforded by NF-kappaB ablation is associated with activation of Akt in mice over-expressing TNF-alpha. *Am J Physiol Heart Circ Physiol* 2006;**290**:H590–598.
43. Wong SC, Fukuchi M, Melnyk P, Rodger I, Giaid A. Induction of cyclooxygenase-2 and activation of nuclear factor-kappaB in myocardium of patients with congestive heart failure. *Circulation* 1998;**98**:100–103.
44. Frantz S, Fraccarollo D, Wagner H, Behr TM, Jung P, Angermann CE, Ertl G, Bauersachs J. Sustained activation of nuclear factor kappa B and activator protein 1 in chronic heart failure. *Cardiovasc Res* 2003;**57**:749–756.
45. Gupta S, Young D, Maitra RK, Gupta A, Popovic ZB, Yong SL, Mahajan A, Wang Q, Sen S. Prevention of cardiac hypertrophy and heart failure by silencing of NF-kappaB. *J Mol Biol* 2008;**375**:637–649.
46. Liao Y, Takashima S, Maeda N, Ouchi N, Komamura K, Shimomura I, Hori M, Matsuzawa Y, Funahashi T, Kitakaze M. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res* 2005;**67**:705–713.

CARDIOVASCULAR FLASHLIGHT

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To kill two birds with one stone: a pleurocentesis that also drained pericardial tamponade in a stubbed patient

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A 27-year-old drug-abuser presented with tachypnoea, sinus tachycardia, and hypotension (systolic blood pressure 86 mmHg). He had been stubbed with >20 table-knife punctures at his left axillary area (*Panel A*) at a street brawl 1 h before. His jugular veins were prominent, he had pulsus paradoxus, and 86% O_2 saturation at blood gases. He was not bleeding externally. Immediate echocardiogram revealed pericardial fluid, probably haemopericardium, with signs of tamponade (mitral inflow Doppler velocities, *Panel B*). On emergent contrast CT scan of the thorax, a large left haemothorax, haemopericardium of at least moderate degree along with pericardial tearing and pneumopericardium, and subcutaneous emphysema were seen (*Panels C* and *D*). The patient's condition was deteriorating. Neither a thoracic nor a cardiothoracic surgeon was available by that time. Fearing the creation of a bleeding conduit between the pericardial space and the drainage system if pericardiocentesis was performed, it was decided first to place a Billow-tube at his left hemithorax. Surprisingly, soon after the procedure, his vital signs improved markedly and his condition gradually recovered. Intubation was avoided. A new CT scan, performed 4 h later without contrast, confirmed a significant drainage of both the pericardial and the pleuritic haematomas (*Panel E*). He was given antibiotics and was transfused with two units of blood. Myocardial or coronary laceration, if any, passed by clinically undetectable. Troponin peaked at 4.3 ng/L. He has had an uncomplicated course since then. Our patient was lucky to survive because the pericardial blood drained into the pleuritic cavity through the artificial pleuropericardial window that had been created by the knife. More so, he was fortunate not to bleed any more despite pleurocentesis, due to spontaneous haemostasis. Pericardiocentesis for acute haemopericardium sets always a problem for caring physicians.